STATISTICAL ANALYSIS PLAN (SAP)

A prospective, open label, pharmacokinetic study of an oral hydroxyurea solution in children with sickle cell anemia (HUPK)

Sponsor: Nova Laboratories Ltd.

Sponsor ref: INV543

Public Database: Clinicaltrials.gov

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Date: 21 Jan 2022

Version: FINAL 1.0

[redacted]

Statistical Analysis Plan (SAP)

Protocol #:	INV543
Protocol Title:	A prospective open label, pharmacokinetic study of an oral hydroxyurea solution in children with sickle cell anemia
Project Code:	NO01SCK
Study Phase:	I/II
Trial Design:	Open-label pharmacokinetic study
Study Drugs:	Oral hydroxyurea (HU) 100mg/mL solution
Patients:	35 (maximum)
Treatment Period:	12-15 months
Sponsor Contact:	Sarah Edwards Nova Laboratories Limited Martin House, Gloucester Crescent, Wigston, Leicester, LE18 4YUL, UK +44 (0) 116 2230100 [redacted]
Analysis Contact:	[redacted]
Date:	January 21, 2022
Status:	Final V.1

[redacted]

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Date of Final Protocol:	20-JAN-2020 (Jamaica-only protocol v. 6.0) 23-MAR-2020 (UK/Global procool v 7.0)
Amendments:	Amendment #1 (Jamaica, 09-APR-18) Amenmdent #2 (Jamaica, 20-DEC-18) Amendment #3 (Jamaica, 07-JUN-19) Amendment #4 (Jamaica, 14-MAY-20) Amendment #5 (Jamaica, TBD) Amendment #1 (UK/Global, 10-DEC-18) Amendment #2 (UK/Global, 12-FEB-19) Amendment #3 (UK/Global, 03-JUN-19) Amendment #4 (UK/Global, Rejected) Amendment #5 (UK/Global, 12-FEB-20) Amendment #6 (UK/Global, 11-MAR-20) Amendment #7 (UK/Global, 16-APR-20) Amendment #8 (UK/Global, 28-JUL-20) Amendment #9 (UK/Global, 18-AUG-20)
Date of Final Plan	21-JAN-2022

I have reviewed the Statistical Analysis Plan. My signature below confirms my agreement with the contents and intent of this document.

Author:

[redacted] [redacted]

[redacted] Date:

Senior Statistician, [redacted]
Report Function: Writer and Statistician

Reviewed by:

[redacted] [redacted]

[redacted] Date:

Report Function: Statistical Reviewer

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Reviewed by:

[redacted]

Hussain Mulla
Head of Clinical and Regulatory
Nova Laboratories Ltd
Report Function: Reviewer

14 Jan 2022
Date:

[redacted]

Sarah Edwards
Clinical Trial Manager
Nova Laboratories Ltd
Report Function: Reviewer

24 JAN 2022
Date:

[redacted]

1. List of Abbreviations Definition of Terms

Abbreviation or Term	Definition
ACR	Albumin to creatinine ratio
AE	Adverse Event
ANC	Absolute neutrophil count
APR	Analysis Programming Requirements - detailed programming specifications required to convert the CRF data into analysis/presentation data sets
ARC	Absolute reticulocyte count
AUC	Area under the plasma concentration time curve
CL/F	Clearance
C_{max}	Maximum plasma concentration
CTCAE	Common Terminology Criteria for Adverse Events
DMP	Data Management Plan - details of how data are managed throughout the trial
EDC	Electronic Data Capture (i.e. eCRF)
eGFR	Estimated Glomerular Filtration Rate
EMA	European Medicines Agency
FDA	(United States) Food and Drug Administration
Hb	Hemoglobin
HbF	Fetal hemoglobin
HU	Hydroxyurea
LDH	Lactate dehydrogenase
LFT	Liver function test
LNH	Low/normal/high
MCH	Mean Corpuscular Hemoglobin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities (coding for AEs)
PK	Pharmacokinetics
SAP	Statistical Analysis Plan
SCA	Sickle cell anemia
SI	International System of Units
$t_{½}$	Half-life
T_{max}	Time to maximum concentration
V/F	Volume of distribution
WBC	White blood cell count

[redacted]

Abbreviation or Term	Definition
WHODD	World Health Organization Drug coding Dictionary managed by the Uppsala Monitoring Centre

[redacted]

2. Background

INV543 is a prospective, open-label safety and pharmacokinetics study of a novel oral solution of hydroxyurea (HU) in the treatment of sickle cell anemia (SCA) in pediatric populations. Hydroxyurea is an established disease-modifying agent in patients with SCA that induces expression of fetal hemoglobin (HbF). The US Food and Drug Administration (FDA) approved hydroxyurea for the treatment of adults with SCA in 1997. The European Medicines Agency (EMA) approved it for treatment of both children and adults with SCA in 2007. The liquid version used in this study was licensed as Xromi for use in >2 years of age in the European Union in 2019 and the UK in 2021.

Pediatric (<18 year old) subjects will be started on a starting dosage of 15mg/kg/day of novel oral HU solution, with the possibility of dose escalation if the current dosage does not display a lack of tolerability in its safety and if there is a failure to achieve mild neutrophil and reticulocyte suppression. Patients will remain in the study for up to 15 months for dose escalation and routine follow-up, with appointments bi-weekly for the first two months and then monthly thereafter.

3. Objectives

The aim of INV543 is to investigate the pharmacokinetics, safety, palatability, and acceptability of oral hydroxyurea 100mg/mL solution in patients with SCA aged from 6 months to 17.99 years (i.e. to the day before the 18th birthday) who, in the clinical opinion of the investigators, would benefit from hydroxyurea treatment.

3.1. Primary Objective(s)

To determine pharmacokinetics of oral hydroxyurea solution (a population pharmacokinetic approach will be adopted to characterize the PK in this population).

3.2. Secondary Objective(s)

- To further evaluate the safety of oral hydroxyurea solution
- To assess the effects of oral HU solution on fetal hemoglobin (HbF), hemoglobin (Hb), mean corpuscular volume (MCV), white blood cell count (WBC), absolute neutrophil count (ANC), absolute reticulocyte count (ARC), platelets, bilirubin, cystatin C, and lactate dehydrogenase (LDH)
- To assess effects of oral HU solution on pain, transfusions, hospitalizations, and acute chest syndrome
- To assess the acceptability and palatability of oral hydroxyurea solution
- To investigate the effects of HU dose escalation on laboratory and clinical parameters

[redacted]

3.3. Safety Objective(s)

To evaluate the safety of oral HU solution, including the incidence of adverse events.

4. Study Design

INV543 is an open-label, observational safety and pharmacokinetic study of oral HU solution in SCA patients aged 6 months to 17.99 years (i.e. to one day before their 18th birthday). Children presenting at the hospital clinic for review or treatment of SCA will be assessed by the study investigator to determine whether the child might benefit from HU treatment. If the physician feels the patient would benefit from HU treatment and the parent(s)/legal guardian and child (where applicable) provide consent.

Subjects will begin on a dose of 15 mg/kg/day oral HU (100 mg/mL solution). After 8-12 weeks, dependent upon clinical and laboratory results (see Protocol section 1.3.3), subjects' dosage may be increased by 5 mg/kg/day every 8-12 weeks (not to exceed 35 mg/kg/day) if subject has not reached the target absolute neutrophil count of 1-3 x 10^9 /L while maintaining platelet count >80 x 10^9 /L. During active pandemic periods of COVID-19, dose escalations will be avoided as possible, only to be carried out per protocol or per investigator discretion when sufficient infrastructure is in place within the hospital/clinic or central laboratories to ensure subject safety. Due to the possibility of extended periods (12 weeks) between clinic visits during the pandemic, investigators are also authorized to treat signs of toxicity proactively and engage dose reduction even before the strict toxicity benchmark is achieved.

Two PK visits are scheduled: on study day 1 and in Weeks 20-36. Subjects will have a pre-dose blood draw and 2-5 post-dose samples taken, depending on subject age and investigator discretion. Post-dose samples will be taken at the following selected times (hours): 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, and 6. Additional single blood samples for PK will be taken at interim clinic visits, where feasible. Subjects will remain on treatment until Week 60 or early study withdrawal; 4 weeks after stopping treatment, subjects will receive follow-up by phone call.

4.1. Primary Outcomes

The following pharmacokinetic endpoints, calculated at the two PK visits separately (Day 0 and Week 20-36) are the primary outcomes:

- Clearance (CL/F)
- Volume of distribution (V/F)
- Time to maximum concentration (T_{max})
- Maximum plasma concentration (C_{max})
- Area under the plasma concentration time curve (AUC)
- Half-life ($t_{½}$)

[redacted]

4.2. Secondary Outcomes

Since this study is focused on PK (primary outcomes) and safety, the following safety endpoints are considered secondary outcomes:

- Incidence of adverse events
- Absolute neutrophil count
- White cell count
- Platelets
- Elevation in liver function tests (LFTs)
- Hemoglobin/anemia
- Additional safety laboratory parameters
- Bacterial infections
- Viral infections
- Fungal infections
- Leg ulcers

Additionally, the following biomarker endpoints are also secondary outcomes:

- Fetal hemoglobin
- Hemoglobin
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Hemoglobin (MCH)
- Cystatin C
- Estimated Glomerular Filtration Rate (eGFR)

Finally, the following clinical status endpoints are also secondary outcomes:

- Incidence of acute pain crises
- Number and frequency of blood transfusions
- Incidence of acute chest syndrome
- Dose escalations required to reach maximum tolerated dose
- Clinical parameters (symptoms)
- Parent/caregiver acceptability/usability by questionnaire
- Palatability and Acceptability: evaluation of taste acceptability

4.3. Exploratory Outcomes

- Transcranial Doppler velocity
- Urine parameters (albumin, creatine, and albumin to creatinine ratio (ACR))
- Incidence of hospitalizations post-treatment compared to hospitalizations in 12 months prior to treatment

[redacted]

5. Data Management

5.1. Data Management

Data will be collected at the sites via an electronic data capture (EDC) system. The study-specific application will be developed based on the protocol requirements and following the full Systems Development Lifecycle (SDLC). The development and management of the trial application, including security and account administration, will adhere to the Standard Operating Procedures (SOPs) at [redacted] All clinical research staff will be trained in the use of the application, and the training documented prior to each site being initiated.

The application design will, where appropriate, provide choice fields in the form of checkboxes, buttons and lists to aid in ensuring high quality standardized data collection. In addition, Data Logic Checks (or data Edit Checks) will be built into the application based on variable attributes (e.g. value ranges), system logic (e.g. sequential visit dates) and variable logic (e.g. onset date must be before cessation date). Visual review and data responses will be overseen by a trained data manager.

Pharmacokinetic modelling and parameter calculation will be performed by [redacted]. A data transfer agreement has been prepared and finalized for the exchange of relevant data (including pharmacokinetic concentrations and laboratory results) to and from [redacted]. Additional data transfer agreements for raw pharmacokinetic data from [redacted] and central lab data from [redacted] have also been finalized.

The database will be locked when all the expected data has been entered into the application, all query responses have been received and validated, the designated data have been noted as monitored in the system and each investigator has signed off the casebook for each of their study subjects. The data coding must be accepted by the Sponsor, or the Sponsor delegate, and any Serious Adverse Events (SAEs) reconciled with the pharmacovigilance database working with the Medical Monitor. Severity of adverse events will be assessed by investigators using Common Terminology Criteria for Adverse Events (CTCAE) version 4.

The data management processes are outlined in the project specific Data Management Plan (DMP); this and all related documentation are on file at [redacted] and are identified by the project code NO01SCK.

5.2. Coding

The adverse events were coded in MedDRA version 23.0 and signed off by a medical monitor designated by the Sponsor or the Sponsor's delegate. All concomitant medication was coded using WHO Drug Global (version 01 Sep 2021) and reviewed and signed off prior to data base lock.

[redacted]

5.3. Missing Data

Data will be presented as observed and entered into the EDC. No imputation will be performed for missing data for any non-pharmacokinetic analyses. Pharmacokinetic analyses and associated missing data handling are described in the Pharmacokinetic Modeling and Simulation Analysis Plan (version 1, 29MAY20)

6. Change to Analysis as Outlined in the Protocol

The protocol states that two interim analyses were planned for this study. Due to changes in subject enrollment rate and study timeline, these interim analyses were determined to be unnecessary and were not performed.

7. Statistical Methods

7.1. Study Populations

7.1.1. Pharmacokinetic Population

The Pharmacokinetic population consists of all subjects who have received at least one oral dose of hydroxyurea and who have at least one valid plasma PK measurement. Further specifics regarding the PK population are found in the Pharmacokinetic Modeling and Simulation Analysis Plan.

The PK population will be used for all analyses of primary outcomes/PK modeling.

7.1.2. Safety Population

The Safety population consists of all subjects who receive at least one dose of study medication. This population will be used for all analyses of secondary and exploratory outcomes.

7.2. Calculated Outcomes

The following are key endpoints derived from data captured at the sites via the EDC system. Complete documentation of the calculations and data manipulation required to go from the EDC database to the analysis database are contained in the companion document - the study Analysis Programming Requirements (APR). For laboratory values, body measurements, and vital signs, measurements will be converted to standardized preferred units for summaries. Listings will display both recorded measurements and standardized.

Baseline Value: Baseline values for any measurement are defined as the last non-missing measurement prior to the first exposure to oral HU.

Change from Baseline: Value at time point – Baseline value.

[redacted]

Time in Trial (days): Date of last contact – Date of informed consent + 1.

Study Day: Study Day is calculated based on the time of first exposure to oral HU, with the first treatment date as Study Day 1.

Treatment Exposure/Time on Treatment: The days between the date when the first dose of study medication intake (Study Day 1/PK Visit 1) and the date of the last medication intake as recorded in the Study Drug Termination form.

Treatment Emergent Adverse Events (TEAEs): An adverse event where the time of onset is on or after the time of the first dose of study medication (Study Day 1/PK Visit 1).

7.3. Interim Analysis

There will not be any interim analyses for this study (see section 6).

7.4. Analysis Methods

The pharmacokinetic modelling and PK parameter estimation for the primary outcomes will be performed by [redacted] The specifics of these analyses are thus outside the scope of this SAP and will be detailed in the Pharmacokinetic Modeling and Simulation Analysis Plan.

All calculations and analyses will be performed using SAS version 9.4 or higher resident on the Windows Server 2012R2 at [redacted] The continuous data will be summarized with N, mean, standard deviation, median and range, while the categorical data will be presented as counts and percentages (or proportions) for the descriptive displays.

8. Results

8.1. Study Subjects

All data collected will be at a minimum listed.

Summarization of continuous data will include n, mean, median, standard deviation, coefficient of variation, minimum, and maximum. Summarization of categorical variables will consist of the number of percentage of patients in each category.

8.1.1. Patient Disposition

All subjects whose parent(s)/legal guardian(s) signed informed consent will be summarized. All early discontinuations will be summarized by reason for discontinuation and by treatment dose at the time of discontinuation. Summaries will be presented for all subjects overall and by age category (6 months to 1.99 years, 2 years to 5.99 years, and 6 years to 17.99 years).

[redacted]

Treatment exposure (time in trial and time on treatment) will be summarized by treatment dose and overall. Overall exposure (total number of days on treatment minus the number of missed doses) will also be summarized.

8.1.2. Patient Characteristics

8.1.2.1. Baseline Characteristics

Demographic (age, sex and race) and baseline vital signs (height/length, weight, BMI, heart rate, respiratory rate, blood pressures) will be summarized overall and by age category. All screening data will also be listed.

8.1.2.2. Medical History

A listing of the abnormal medical history will be presented by patient including year of onset/ended and current ongoing (Yes or No) status. Acute complications (as listed in protocol section 5.5) and hospital admissions in the 12 months prior to screening will be summarized by subject frequency, overall and by age category. SCA and non-SCA medical histories will be presented separately.

8.2. Primary Outcomes

The population pharmacokinetic parameters will be estimated and reported separately by [redacted] As such, these analyses are detailed by the separate Pharmacokinetic Modeling and Simulation Analysis Plan.

8.3. Secondary Outcomes

8.3.1. Laboratory Outcomes

The following secondary endpoints will be summarized by visit, both overall and by age category:

- White blood cell count (WBC)
- Platelets
- Fetal hemoglobin (HbF)
- Hemoglobin (Hb)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Absolute neutrophil count (ANC)
- Absolute reticulocyte count (ARC)
- Total bilirubin
- Cystatin C
- Lactate dehydrogenase (LDH)

[redacted]

Summaries will include changes from baseline and percent change from baseline in these measurements at each assessment visit. Shift tables will be provided to show the change from baseline low/normal/high (LNH) laboratory results to the LNH results at each scheduled visit.

These and select other laboratory values will be represented graphically in two ways: box-and-whisker plots will be provided for specified timepoints by age category. Additionally, line charts of arithmetic means over time will be provided that include all age categories and overall in a single chart per lab analyte.

A table summarizing incidence of hematological toxicity by visit and type will also be provided, by age category and overall.

8.3.2. Infections and Clinical Symptoms

Incidence of the following infections and clinical symptoms will be summarized by visit, overall, and by age category:

- Bacterial infections
- Viral infections
- Fungal infections
- Leg ulcers
- Acute pain crises
- Blood transfusions
- Acute chest syndrome
- Chronic pain
- Daytime somnolence
- Enuresis
- Painful crises
- Shortness of breath on exertion
- Snoring
- Stuttering priapism

8.3.3. Dose Escalations/Maximum Tolerable Dose

Maximum tolerable dose/dose at the end of study or early termination will be summarized overall and by age category. Incidence of subject dose de-escalation may be summarized depending on the frequency of this occurrence; dose escalations and de-escalations will also be captured in listings. Figures of dose over time (in mg/kg) will be presented by age category and overall. Proportion of patients achieving maximum tolerable dose over time will also be presented graphically by age category and overall.

[redacted]

8.3.4. Palatability and Acceptability

Assessments of palatability and acceptability of the oral HU solution will be summarized overall and by subject age category. Parental assessments and subject assessments (where applicable) will be summarized separately as well as in a pooled summary across assessment type. Questions will be summarized by count and percent of subjects/parents choosing each categorical response. Transcriptions of verbal comments or other qualitative data will be captured in the listings. Ratings of taste, smell, and aftertaste will also be presented in a histograms for visual summary.

8.4. Exploratory Outcomes

The following measurements will be summarized by visit, overall and by age category in regions where they are available.

- Urine albumin
- Urine creatinine
- Urine albumin to creatinine ratio (ACR)

8.4.1. Transcranial Doppler Velocity

Transcranial Doppler Velocity will be summarized by, for each subject, obtaining the maximum time-averaged mean maximum (TAMx) velocity across all vessels and both sides. These maximum TAMx values will be summarized at Screening and EOS. Additionally, incidence will be summarized at Screening and EOS of subjects falling into "Normal" (< 170 cm/s), "Conditional" (170-199.9 cm/s), and "Abnormal" (≥ 200 cm/s) maximum TAMx values. Shift tables will show subjects in each category moving from one category to another from Screening to EOS.

8.4.2. Hospitalizations

The overall number of hospitalizations in the 12 months prior to the study will be presented by four physician-determined categories: Acute Chest Syndrome, Vaso-occlusive episode, Other SCA-related, Other. This will be presented by age group and overall. Hospitalizations in the 12 months post-treatment will also be summarized by age group and overall. Average hospitalizations per patient-month will be summarized by age category and overall for the 12 months prior to screening and the 12 months after treatment. The difference in average hospitalizations per patient-month will be summarized with a 95% confidence interval; this is exploratory and not intended to be generalizable.

8.5. Safety Outcomes

The safety profile will primarily be analyzed by means of descriptive statistics and qualitative analysis. All summaries and listings in the section are based on the Safety population.

[redacted]

8.5.1. Adverse Events

The adverse events reported will be summarized using the counts of patients and also the number of reports, for each SOC and Preferred term. The presentations will separate out and highlight any Serious Adverse Events (including deaths) and adverse events leading to discontinuation from the study

The summaries will at a minimum be: 1) the number of patients reporting for all events; 2) the number of reports for all events; 3) the number of patients reporting treatment-related events (probable and possible relationship); 4) the number of reports of treatment-related events.

Summaries of adverse events by severity and by relatedness to oral hydroxyurea will also be provided.

A by-patient listing of all adverse events, with study time based on the first administration of study drug.

8.5.2. Concomitant Medication

The concomitant medications will be listed by patient; the reason for the medication as well as the start and stop date/time will be presented. Concomitant medications will also be tabulated and summarized by anatomical system and therapeutic class overall and by age category.

8.5.3. Vital Signs

Vital signs (temperature, heart rate, respiration, systolic blood pressure, and diastolic blood pressure) and body measurements (weight and height/length) will be summarized as mean values with variance, including the change from baseline, overall and by age category.

8.5.4. Physical Examinations

Any abnormal results from the physical examinations performed at screening and Week 24 visits will be listed by subject.

8.5.5. Compliance and Drug Accountability

Drug accountability and compliance data, as recorded in the subject's drug diary and transcribed into the EDC, will be listed. Summaries of missed doses between visits will be presented overall and by age category.

8.5.6. Laboratory Analytes

For laboratory analytes that are not secondary outcomes, the following applies.

Haematology and biochemistry data will be presented as mean values with variability and change (absolute and relative) from baseline in each laboratory parameters. Results will be summarized overall and by age category.

[redacted]

Results for urinalysis visit will be summarized by dose cohort.

8.5.7. Pregnancy Testing

All information regarding subjects' childbearing potential and any pregnancy tests and/or pregnancies will be listed.